PTO 08-1059

A RHODOSPIRILLUM SP. PREPARATION AND THE PREPARATION METHOD AND APPLICATION THEREOF
[Hong Luo Jun Zhi Ji ji Qi Zhi Bei Fang Fa he Ying Yong]

HOUDE WANG, et al.

UNITED STATES PATENT AND TRADEMARK OFFICE Washington, D.C. November 2007

Translated by: FLS, Inc.

PUBLICATION COUNTRY	(19):	CN
DOCUMENT NUMBER	(11):	1172653
DOCUMENT KIND	(12):	A
PUBLICATION DATE	(43):	19980211
APPLICATION NUMBER	(21):	97112215.6
DATE OF FILING	(22):	19970715
ADDITION TO	(61):	
INTERNATIONAL CLASSIFICATION	(51):	A61K 35/74; C12P 1/04; C12N 1/20
PRIORITY	(30):	
INVENTORS	(72):	WANG, HOUDE; LI, SHENGXUE
APPLICANT	(71):	LI, SHENGXUE
DESIGNATED CONTRACTING STATES	(81):	
TITLE	(54):	A RHODOSPIRILLUM SP. PREPARATION AND THE PREPARATION METHOD AND APPLICATION THEREOF
FOREIGN TITLE	[54A]:	HONG LUO JUN ZHI JI JI QI ZHI BEI FANG FA HE YING YONG

- 1. A rhodospirillum sp. preparation, wherein it is characterized by using three rhodospirillaceae genera:
  - (1) rhodospirillum
  - (2) rhodopseudomonas
  - (3) rhodomicrobium

of which, any one or multiple of the three are bacteria which are cultured on a primary bacteria, secondary bacteria, or ternary culture medium and added to a small amount of food additive to form a liquid preparation; of which, inject the ternary culture medium with rice flour, corn flour, milk powder, wheat bran, sugar, inorganic salt, trace elements, and vitamins make the base material of which is then cultured, dried, and ground to form a solid preparation.

- 2. A preparation method for the liquid and solid rhodospirillum sp. preparation as described in Claim 1, being:
  - (A) preparation of the liquid preparation:
  - (1) primary bacteria preparation:

prepare beef extract or yeast extract as one group; potassium dihydrogen phosphate  $(KH_2PO_4)$ , magnesium sulfate  $(MgSO_4 \cdot 7H_20)$ , sodium acetate  $(CH_3CooNa)$ , and sodium chloride (NaCl) as one group; and sodium carbonate  $(Na_2CO_3)$  as one group to form a total of three groups as mediums; separately sterilize in human heated tap water at high pressure (0.14 MPa) for 30 seconds; compensate with sterile water to the required amount after cooling; of which, the volume percentage of each ingredient in the

<sup>\*</sup> Numbers in the margin indicate pagination in the foreign text.

water solution is:

beef extract or yeast extract 0.01%

potassium dihydrogen phosphate 0.03 - 0.05%

magnesium sulfate 0.01%

sodium acetate 0.2 - 0.4%

sodium chloride 2%

sodium carbonate 0.1 - 0.2%

then, mix the three groups together; under sterile conditions, inject 5% (the following is a volume percentage) bacteria; the bacteria are of three rhodospirillaceae genera:

rhodospirillum

rhodopseudomonas

rhodomicrobium

of which, any one or multiple of the three are bacteria; sterile culture for 2 - 3 days at  $28^{\circ} \pm 2^{\circ}\text{C}$  under natural or incandescent light (500 - 2200 lux) to become the primary bacteria; store for future use;

(2) secondary bacteria preparation:

the medium and operation is the same as the primary bacteria; the difference is to inject 10% primary bacteria;

(3) ternary culture medium preparation:

the medium and operation is the same as the primary bacteria; the difference is to inject 20% secondary bacteria;

(4) preparation of the liquid preparation:

in a 100 ml ternary liquid culture medium, 0.01 g saccharin sodium, and 0.001 ml orange essence radio, dissolve the saccharin sodium into

the ternary culture medium; add the orange essence; mix and package;

- (B) preparation of the solid preparation:
- (1) mix 26% rice flour, 24% corn flour, 10% milk powder (skim), 38% wheat bran, 1.8% sugar, and 0.2% inorganic salt and vitamins to form a mixture; use tap water for the base material; sterilize at 0.14 MPa for 1 hour; inject ternary culture (base material: ternary culture medium /2 = 1 : 0.5) after cooling; sterilize at 28° ± 2°C under 500 2700 lux natural or incandescent light conditions and culture for 2 3 days;
- (2) dry: dry < 80°C conditions, the water content of the material
  < 5%;</pre>
  - (3) grind;
  - (4) package.
- 3. A rhodospirillum sp. preparation as described in Claim 1, wherein it is characterized by being applied to treat certain digestive tract diseases; primarily colitis, acute and chronic enteritis, gastritis, gastric ulcer, constipation, acute and chronic hepatitis, cirrhosis, and malignant tumors of the digestive tract.

Description of the Invention

/3

# A RHODOSPIRILLUM SP. PREPARATION AND THE PREPARATION METHOD AND APPLICATION THEREOF

The present invention involves a type of microorganism preparation. Specifically, it utilizes a rhodospirillaceae bacteria preparation and its preparation method and application for the treatment of certain digestive tract diseases.

Microecological modulators currently used to treat digestive tract

disease primarily include difidobacterium, bacillus cereus, bacillus licheniformis, lactobacillus, and streptococcus faecalis. Reports of application of rhodospirillum sp. have yet to be seen. All of the aforementioned bacteria preparations have several drawbacks in the treatment of digestive tract diseases: 1. Treatment is not only unclear, but the preparation can only be an adjuvant therapy agent or preventive agent. 2. The single weight or volume of the cells is too small, typically only 0.1 - 2 billion/g (ml). Treatment is also not very effective because of the low biomass of the cells. 3. The preparation can only be stored for a short amount of time; only half of a year at room temperature. Cell decline exceeds one-half. The liquid preparation can only be stored for 1 - 5 days after the bottle is opened.

The purpose of the present invention is to provide a type of rhodospirillaceae bacteria preparation and the preparation method, as well as application of said preparation to improve the effectiveness of treating certain digestive tract diseases, specifically the effectiveness of treating colitis. Similarly, the unit weight (volume) cell count of said preparation is increased, which extends storage life and ensures the quality of said preparation.

The purpose of the present invention is achieved through the following technical solution:

The rhodospirillum sp. preparation uses three rhodospirillaceae genera:

rhodospirillum

rhodopseudomonas

#### rhodomicrobium

of which, any one or multiple of the three are bacteria which are cultured on a primary bacteria, secondary bacteria, or ternary culture medium and added to a small amount of food additive to form a liquid preparation. Of which, inject the ternary culture medium with rice flour, corn flour, milk powder, wheat bran, sugar, inorganic salt, trace elements, and vitamins make the base material of which is then cultured, dried, and ground to form a solid preparation.

The aforementioned rhodospirillum sp. liquid and solid preparation method and specific operation is:

- (A) Liquid preparation:
- 1. Primary bacteria preparation:
- (a) Culture: beef extract or yeast extract as one group; potassium dihydrogen phosphate  $(K_2H_2PO_4)$  [sic], magnesium sulfate  $(MgSO_4 \cdot 7H_20)$ , sodium acetate  $(CH_3CooNa)$ , and sodium chloride (NaCl) as one group; and sodium carbonate  $(Na_2CO_3)$  as one group.
- (b) Operation: Separately sterilize each component of the three mediums in human heated tap water at high pressure (0.14 MPa) for 30 seconds. Compensate with sterile water to the required amount after cooling. Of which, the volume percentage of each ingredient in the water solution is:

Beef extract or yeast extract 0.01%

Potassium dihydrogen phosphate 0.03 - 0.05%

Magnesium sulfate 0.01%

Sodium acetate 0.2 - 0.4%

Sodium chloride 2%

Sodium carbonate 0.1 - 0.2%

Then, mix the three groups together according to the required ratio.

Under sterile conditions, pack in sterilized 50 - 250 ml conical flasks.

Under sterile conditions, inject 5% (the following is a volume percentage)

of the aforementioned bacteria. Insert a sterile rubber plug. Culture /4

2 - 3 days at 28° ± 2°C under natural or incandescent light (500 - 2200 lux). Wait for the cell count to reach 40 billion/ml before terminating the culture to make the primary bacteria. Store for later use.

### 2. Secondary bacteria preparation:

The medium and operation is the same as the (a) primary bacteria. The difference is to inject 10% primary bacteria. (b) The culturing container is a 500 ml narrow-mouth colorless glass flask. The cell count reaches 30 billion/ml.

### 3. Ternary culture medium preparation:

The medium and operation is the same as the (a) primary bacteria. The difference is to inject 20% secondary bacteria. (b) The culture container is a 1000 - 5000 ml colorless glass flask. Seal with a glass lid. The cell count is required to reach 30 billion/ml.

## 4. Preparation of the liquid preparation:

In a 100 ml ternary liquid culture medium, 0.01 g saccharin sodium, and 0.001 ml orange essence radio, dissolve the saccharin sodium into the ternary culture medium. Add the orange essence. Mix, pour in bottles, seal, and package.

#### (B) Preparation of the solid preparation:

1. Injection and culture:

Mix 26% rice flour, 24% corn flour, 10% milk powder (skim), 38% wheat bran, 1.8% sugar, and 0.2% inorganic salt and vitamins to form a mixture using tap water for the base material. Sterilize at 0.14 MPa for 1 hour. Inject ternary culture (base material: ternary culture medium = 1 : 0.5) after cooling. Place in an enamel plate. Cover with a sterile plastic film. Sterilize at  $28^{\circ} \pm 2^{\circ}$ C under 500 - 2700 lux natural or incandescent light conditions and culture for 2 - 3 days. Wait for the cell count to reach 80 billion/g before terminating the culture.

- 2.) Dry: Dry in a < 80°C electrothermal blast drying chamber or use freeze drying or vacuum drying. The water content of the material is < 5%.
  - 3. Grind: Use a Chinese medicine mill to grind it to a 40 mesh powder.
- 4. Package: Package using a capsule machine and then press in an aluminum board and seal.

The primary technical indicators of the preparation of the present invention are:

- 1. Appearance and look indicators:
- (a) Outward appearance: The liquid preparation is flesh pink and sticky and thick. The solid preparation is a dark brown powder.
- (b) Odor: The liquid preparation has a slight sulfur dioxide odor. The solid preparation has a specific fermentation odor.
  - (c) Taste: A slight sweet and salty tastes.
  - 2. Microbial indicator:

Total cell count: Liquid preparation is > 20 billion/ml, solid

preparation is > 80 billion/g.

. . . . . . . . .

The rhodospirillum sp. liquid state and solid preparations of the present invention can be applied to treat certain digestive tract diseases; primarily used for colitis, acute and chronic enteritis, gastritis, gastric ulcer, constipation, acute and chronic hepatitis, cirrhosis, and acute malignant tumors of the digestive tract. For acute and chronic enteritis, especially colitis, only administration of 1 - 2 periods of treatments is needed for curing. One period of treatment can be 1 - 7 days. The period of treatment depends on the status of the disease. Administer twice a day. Administer 25 ml of the liquid preparation or 4 capsules (each capsule contains approximately 0.32 g of the preparation) each time. Administration can also consist of 25 ml of the liquid preparation with 2 capsules. For gastrointestinal discomfort caused by enterogastritis, effectiveness will be noticed in 5 - 40 minutes. For long-term period of treatment of chronic colitis, only 1 - 3 months of taking the drug is needed to cure the ailment. /5

Observation of 102 clinical treatments at the Second People's
Hospital of Jilin City resulted in a clear effectiveness in the treatment
of digestive tract disease with said preparation. A second observation
of treatment (a treatment period being 6 days) resulted in a total
effectiveness rate reaching 85% without any toxicity.

The primary benefits of the method of the preparation prepared with the present invention:

1. Increase treatment of digestive tract diseases, specifically increasing the effectiveness of treating colitis.

- 2. Increase the preparation single unit weight (volume) cell count.
- 3. Extend the storage time to up to one year, making it easier to transport, store, and ensure product quality.